

# Recent Advances in Mass Clonal Propagation of Teak

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## ABSTRACT

*Tectona grandis*, commonly called teak, is one of the best-known and highest value timbers in the world, with certain specific features that justify investment in clonal forestry. Possibilities for vegetative propagation of mature teak genotypes were assessed by both advanced nursery and tissue culture development-oriented techniques. Nursery experiments established the influence of cutting type on the potential of mature teak trees to be clonally mass-propagated through rooted cuttings, leading to the definition of a standard, which was shown to be better achieved by intensive management of the container-grown stock plants. Concurrently, adapted tissue culture methods applied to a few nodal primary explants demonstrated the possibility to produce within a few months and using a simple basal culture medium, several thousands of *in vitro* plantlets that can be easily acclimatised to outdoor conditions. Such results indicate that clonal propagation can be realistically applied to mature superior genotypes of teak, providing suitable nursery or tissue culture technology is available.

## INTRODUCTION

Teak (*Tectona grandis*) produces one of the highest quality timbers in the world and occurs naturally, though discontinuously, in deciduous forests between latitudes 9°-26° North and longitudes 73°-104° East, a zone that includes central and southern India, Myanmar, northern Thailand and Laos. It has been introduced to many Southeast Asian countries such as Indonesia (especially Java about 400-600 years ago), Sri Lanka, Vietnam and the Solomon Islands. In 1990 the species was reported to cover about 25 millions ha (Dupuy, 1990).

The main aspects of research and development of teak have been well reviewed by White (1991).

Planting stock is still produced from seed, as it has been done traditionally for centuries, despite certain handicaps such as:

- \* quantitatively limited and late seed production (Mascarenhas *et al.*, 1987; White, 1991), bearing in mind that teak of straight bole length, which directly affects its market value, is strictly governed by the ability of the terminal meristem to remain vegetative as long as possible;
- \* low germination rates;
- \* substantial overall variability among individuals within progenies;
- \* lack of accurate knowledge about the inheritance of economically significant traits.

These limitations constitute good reasons to explore the potential for true-to-type mass cloning of teak using macro- and micro-propagation technologies (Kaosa-ard *et al.*, 1987; Mascarenhas *et al.*, 1987). These technologies are a prerequisite to clonal forestry, whose general aspects and benefits have already been widely reported (Zobel and Talbert, 1984; Ahuja and Libby, 1993).

## MATERIALS AND METHODS

### Macropropagation

#### Plant Materials

Five- to six-year-old teak trees (origin: Solomon Islands) were selected from the Luasong Forestry Centre, Sabah, East Malaysia. Some of them had just begun flowering and could be considered mature (Wareing, 1959). Although submitted exactly to the same experimental treatments, the genotypic identity was kept separate for clone numbers 1 to 8, corresponding to the 8 genotypes selected. The stock plants used as source material for these experiments were obtained from newly developed sprouting shoots emerging from the stump after the ortets had been cut at about 20-30 cm above ground level (Monteuuis *et al.*, 1995). The stock plants were kept under shade in plastic bag containers, each filled with 10:1 of local clay:loam top-soil, suitably watered and fertilised monthly with 1.5 g of complete NPK/15-15-15 formulation. The growth of these stock plants was limited to about 20-30 cm in height by frequent hedging and pinching operations. The objective was to stimulate the production of many expanding axillary shoots to be used as top cuttings, with 3 to 6 cm in length from the basal cut to the terminal bud as the only limitation for choosing cuttings for the first experiments. More attention was given to the morphological characteristics of the cuttings used for the second experiments, leading to the definition of two types, "Type 1" and "Type 2", with distinctive traits summarised in Table 1.



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**Table 1.** Main distinctive traits between "Type 1" and "Type 2" teak terminal shoot cuttings

Type 1	Type 2
Flexible herbaceous and pubescent stem	Firm, vigorous, glabrous stem, with big section and quadrangular aspect featured by strong angular ribs
Average number of 2.5 visible nodes per 5 cm long cutting	Low node density, with around 1.5 visible nodes per 5 cm long cutting
Short internodes, about 2 cm in length for the basal one	Long internodes, 4 cm in length for the basal one
More compact morphology	Slender overall development
Short expanding leaves with reduced lamina	Big leaves with large lamina
Soft green colour	Pronounced pigmentation.

### Cutting Propagation

Once selected, and with leaf, the area of large leaves reduced by 50 % - mainly for the first experiment cuttings - to lower the evapotranspiration rate (Hartmann *et al.*, 1990), the bases of cuttings were dipped into Seradix 3 (a commercial root promoting powder preparation containing 0.8 % of indole-3-butyric acid). The cuttings were then set in rooting beds filled with wet sand used as rooting substrate after the sand had been boiled to reduce disease risks. The environmental conditions consisted of 50% shade with intermittent mist water sprays provided during day time by a mist system. The spraying frequency was controlled by an "electronic-leaf" system (Hartmann *et al.*, 1990) to avoid any hydric stress damage. The cuttings were sprayed weekly with aqueous fungicide solutions, mainly "Thiram 80", at 5 g l<sup>-1</sup>.

After a rooting period of two months in these conditions, the capacity for adventitious rhizogenesis was assessed by recording

- the percentage of rooted cuttings;
- the measurement (in cm) of the longest root formed per rooted cutting;
- the number of adventitious roots per rooted cutting.

### Micropropagation

The plant material used for tissue culture consisted of a mixture (bulk) of the above-mentioned clones, which can be considered as a polyclonal Solomon Island variety. Nodal portions (length 1 cm) of elongating shoots, longitudinally split when too big, were used as primary explants after disinfection by immersion for 30 min in 2.5 % HgCl<sub>2</sub> followed by three thorough rinses in sterile distilled water. These explants were then inoculated individually and under sterile conditions in culture test tubes containing 8 ml of a basal medium previously autoclaved at 120 °C for 20 min. Axillary sprouting shoots (0.5 - 1 cm) produced by the responsive primary explants were excised to be transferred

in contamination-free conditions into test tubes, then into culture flasks (6 shoots per flask) using the same basal medium. The microshoots were subcultured following the same procedure on fresh basal medium in flasks every 1.5 months, and the following scores were recorded:

- percentage of mortality;
- multiplication rate obtained by dividing the number of new shoots that constituted the coming S<sub>n</sub> subculture cycle by the number of shoots used 1.5 months before for the S<sub>n-1</sub> subculture, regardless of any defect due to mortality or contamination that might have happened meanwhile;
- rooting percentage based on the number of rooted shoots out of the total still alive at the end of the 1.5 months subculture period.

Data corresponded to average means based on several sample records, each including at least 100 microshoots.

Acclimatisation of the microshoots was carried out using the same mist system conditions as previously described for rooting the cuttings. Percentage of success was assessed after 2 months by recording the number of growing plantlets out of the microshoots initially transferred from *in vitro* conditions.

### RESULTS

The first experiments established that the mature genotypes selected could be propagated through rooted cuttings with average rooting rate of 70%. Figure 1 shows the overall differences in terms of rooting rate, root length and number of newly formed roots that might exist between the various clones tested. Clonal potential for adventitious rooting was found to vary considerably depending on the collection date.

Time factor influence was further investigated considering the effect of different time intervals between two cutting collections - defined as periodicity - on the annual average yield of cuttings produced per stock plant. The relevant data reported in Figure 2 demonstrate that the shortest periodicities generally gave the best productivity scores. On average, around 20 rooted cuttings from mature selected clones could be expected annually per stock plant when collected every three weeks, based on overall rooting rates of 52 %.

However, such a productivity can be greatly improved through higher rooting rates when more attention is paid to cutting selection, as illustrated in Figure 3. After 42 days of rooting period, more than 95 % of "Type 1" cuttings had rooted, as against 46 % for "Type 2" cuttings. The type of cuttings was shown to influence significantly (p<0.001) not only the rooting percentage, but also the length of the longest root and the number of newly formed adventitious roots per rooted cutting.

On average 20 to 30 % of the nodal explants introduced into tissue culture conditions gave rise to contamination-free sprouting axillary shoots. Further *in vitro* manipulations of these shoots according to the procedure described resulted in exponential multiplication rates of 3-4 every 1.5 months, with insignificant mortality and degeneration to date (this experiment has been ongoing for 2 years up to now); 60 to 70 % of the microshoots rooted spontaneously in the basal medium.

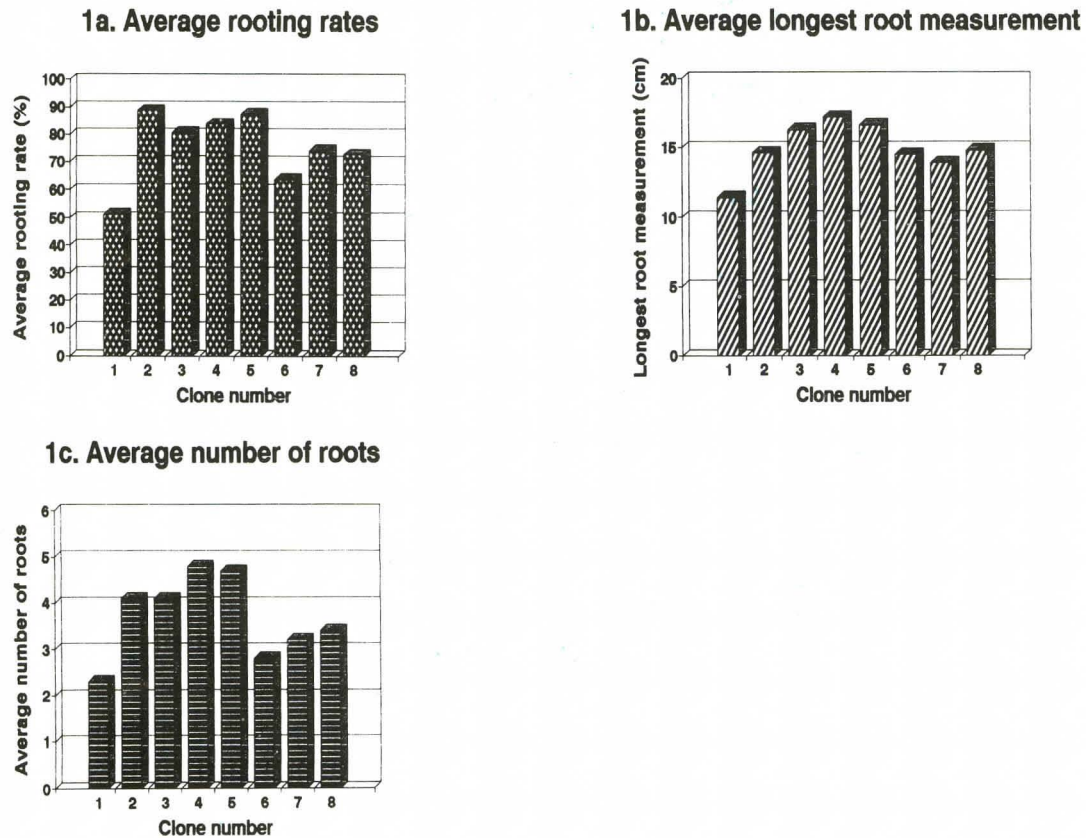


Figure 1. Comparative clonal scores of rootability for cuttings collected from stock plants issued from 6-year-old teak genotypes (first group of experiments), with regard to rooting rate (1a), longest root measurement (1b) and number of adventitious roots (1c) formed per rooted cutting after a rooting period of two months. Average scores were established from 19 different date collections.

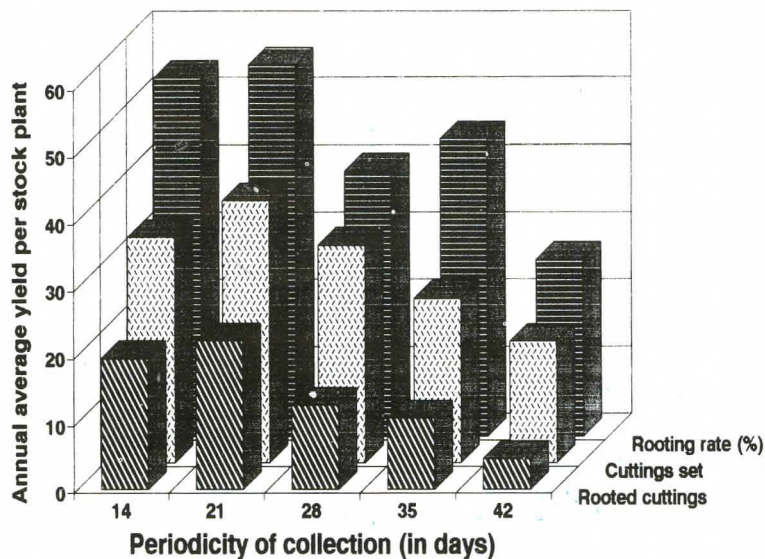
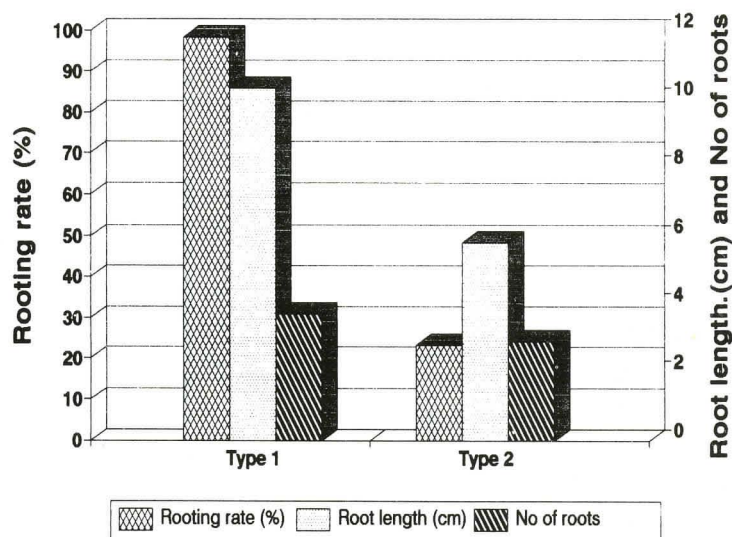


Figure 2. Annual average yield of cuttings produced and set, and cuttings rooted - with resulting rooting rate - per stock plant derived from 6-year-old teak genotypes, for different time intervals between two cutting collection/pinching combined operations - referred to as "periodicity" - (first group of experiments)





**Figure 3.** Comparative potential for adventitious rooting of "Type 1" and "Type 2" (see text and Table 1 for definition) cuttings from stock plants derived from 6-year-old teak genotypes, with regard to rooting rate, longest root measurement and number of roots formed per rooted cutting after a rooting period of 42 days

It has to be mentioned that even the microshoots not rooted *in vitro* can be successfully acclimatised to *in vivo* conditions. More specifically, several thousands of these microshoots were easily acclimatized to outdoor conditions with more than 95 % success rate, regardless of the presence or absence of adventitious roots formed *in vitro*, to develop rapidly into vigorous and true-to-type vegetative offsprings.

## DISCUSSION

The results obtained from these experiments indicate that mature selected teak genotypes can be clonally mass-propagated through rooted propagules using appropriate nursery or tissue culture techniques.

The efficiency of the macropropagation techniques depends on three factors:

- The availability of appropriate propagation facilities including a proper mist system to get the cuttings rooted in the best conditions;
- The characteristics of the cuttings, leading to the definition of a standard, referred to as "Type 1" as defined in Table 1, that really display a greater potential for adventitious rooting;
- The management of the container-grown stock plant, combining judiciously with feeding-watering-hedging-pinching operations to maximise the productivity of "Type 1" cuttings, the frequency of which can be noticeably increased, for instance, by clipping back every three or four days any actively growing vigorous shoots evolving into undesired "Type 2".

Realistic assessments based on experimental data, established that 40 rooted cuttings on average can be produced annually per stock plant, that correspond to 600

rooted cuttings per square meter (15 stock plants per square meter), providing such container-cultivated stock plants can be intensively managed according to the above mentioned precepts.

Lastly, although not tested yet in our experimental conditions, it can logically be assumed that the capacity of the stock plants to produce large amount of cuttings of the desired type will also depend on the nature of the substrate used to fill the containers as it is known to have an effect on root system characteristics and most likely on the physiology of the stock plant.

Compared to literature procedures (Mascarenhas *et al.*, 1987), the tissue culture technology used for propagating mature clones of teak was deliberately kept as simple as possible to cope with the constraints of large scale application, that inevitably implies great productivity and low cost. The methodology was therefore reduced to the use of a sole basal culture medium suitable for multiplication, elongation and, to some extent, adventitious rooting that occurred spontaneously. The benefit of a mist system needs to be emphasised to avoid acclimatisation problems, especially in countries with a dry season (Kaosa-ard and Apavatjirut, 1988). It appeared useless and uneconomical to transfer the microshoots onto specific *in vitro* rooting media as proposed by Gupta *et al.* (1980), because 95 % of them, regardless of whether they had been rooted *in vitro*, could be successfully acclimatised to outdoor conditions.

Considering the good performances of both the macro- and micropropagation techniques presented for mass producing true-to-type rooted propagules of mature selected clones of teak, the economic consideration seems objectively to be the most decisive factor to favour one of these two ways of propagation, which can also be judiciously combined. Although cost comparison requires very

accurate information and data, it can be assumed that the *in vitro* option of producing mature clones will be more expensive than macropropagation (Mascarenhas *et al.*, 1987; Haines, 1994). In order to minimise the production costs, micropropagation can therefore be rationally restricted to outstanding genotypes recalcitrant to horticultural means of mass clonal propagation. Another option is to utilise tissue culture technology to produce only a limited number of genetically superior stock plants with improved potential for vegetative reproduction through rooted cuttings. These can then be used for cheaper clonal mass propagation in nursery conditions while diluting the initial *in vitro* production cost. Research and experiments are in progress aimed at optimising and rationalising these different steps while taking into consideration cost effectiveness at the same time. Meanwhile, the techniques described in this paper can profitably be used to start clonal plantations of mature selected genotypes of teak.

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## REFERENCES

- AHUJA, M.R. AND LIBBY, W.J. (1993) Clonal Forestry. I. Genetics and Biotechnology, 277 pp. Clonal Forestry. II. Conservation and Application, 240 pp. Springer Verlag, Berlin, Heidelberg, New York, London, Paris, Tokyo, Hong Kong, Barcelona, Budapest.
- DUPUY, B. (1990) Notes de voyage en Chine tropicale lors d'un séminaire régional sur le teck. Bois et Forêt des Tropiques 226 : 69 - 76.
- GUPTA, P.K., NADGIR, A.L., MASCARENHAS, A.F. AND JAGANNATHAN, V. (1980) Tissue culture of forest trees: clonal multiplication of *Tectona grandis* L. (Teak) by tissue culture. Plant Science Letters 17 : 259 - 268.
- HAINES, R. (1994) Biotechnology in Forest Tree Improvement. FAO, Rome. 230 pp.
- HARTMANN, H.T., KESTER, D.E. AND DAVIES, F.T. (1990) Plant Propagation: Principles and Practices. 5th edit. Prentice Hall, New Jersey. 647 pp.
- KAOSA-ARD, A., APAVATJRUT, P. AND PARATASILPIN, T. (1987) Teak (*Tectona grandis* Linn. f.) tissue culture. Paper presented at "His Majesty's Fifth Cycle Commemorative Conference of USAID Science Research Award" held in Nakorn Prathom, Thailand, July 24-26, 1987. 9 pp.
- KAOSA-ARD, A. AND APAVATJRUT, P. (1988) Teak (*Tectona grandis* Linn. f.) tissue culture: rooting and transplanting techniques. Paper presented at the PSTC Conference on Biotechnology for Health and Agriculture held in Washington DC, U.S.A., June 6-9, 1988. 12 pp.
- MASCARENHAS, A.F., KENDURKAR, S.V., GUPTA, P.K., KHUSPE, S.S. AND AGRAWAL, D.C. (1987) Teak. In Cell and Tissue Culture in Forestry. Vol. 3. (Bonga, J.M. and Durzan, D.J., eds.). Martinus Nijhoff Publishers, Dordrecht, Boston, Lancaster : 300 - 315.
- MONTEUUIS, O., VALLAURI, D., POUPARD, C., HAZARD, L., YUSOF, Y., WAHAP, GARCIA, C. AND CHAUVIERE, M. (1995) Propagation clonale de tecks (*Tectona grandis*) matures par bouturage horticole. Bois et Forêts des Tropiques (in press).
- WAREING, P.F. (1959) Problems of juvenility and flowering in trees. Journal of the Linnean Society of London 56 : 282 - 289.
- WHITE, K. (1991) Teak: Some Aspects of Research and Development. FAO Regional Office for Asia and the Pacific (RAPA), Bangkok. 53 pp.
- ZOBEL, B. AND TALBERT, J. (1984) Applied Forest Tree Improvement. John Wiley and Sons, New York, Chichester, Brisbane, Toronto, Singapore. 505 pp.